Assessment of Piperacillin-Tazobactam-Meropenem Synergy against Serine Carbapenemase-Producing *Enterobacterales* Using Time-Kill Assays

Alexander Lawandi,^a Gleice C. Leite,^b Matthew P. Cheng,^{c,d} Brigitte Lefebvre,^e Jean Longtin,^e Todd C. Lee^{c,f}

^aDepartment of Critical Care Medicine, National Institutes of Health Clinical Center, Bethesda, Maryland, USA

^bResearch Institute, McGill University Health Centre, Montreal, Quebec, Canada

^cDivision of Infectious Diseases, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada

^dDivision of Medical Microbiology, Department of Laboratory and Pathology Medicine, McGill University Health Centre, Montreal, Quebec, Canada

^eLaboratoire de santé publique du Québec, Sainte-Anne-de-Bellevue, Quebec, Canada

^fClinical Practice Assessment Unit, Department of Medicine, McGill University, Montreal, Quebec, Canada

ABSTRACT Synergy between piperacillin-tazobactam and meropenem against KPC-producing *Klebsiella pneumoniae* was recently demonstrated. We sought to test the combination against a broader range of serine carbapenemase producers. We tested the combination against 10 KPC-producing *Escherichia coli* and 10 OXA-48 family-producing *K. pneumoniae* isolates. Antibiotic concentrations used are achievable in critically ill patients. The combination was synergistic against 7 of 10 KPC producers and 9 of 10 OXA-48 producers. There was no synergy detected in control isolates producing NDM-1.

KEYWORDS *Enterobacterales*, antimicrobial resistance, carbapenemase, meropenem, piperacillin-tazobactam, synergy, time-kill assay

The global spread of multidrug-resistant bacteria is a significant threat to human health (1–3). Carbapenemase-producing *Enterobacterales* (CPE) are of particular concern because infections with these organisms are associated with considerable morbidity and mortality and can be challenging to treat because of limited therapeutic options (4–6). Based on the concept of double-carbapenem therapy (7), we recently investigated other beta-lactam combinations for *in vitro* synergy against KPC-producing *Klebsiella pneumoniae* and found that the combination of piperacillin-tazobactam and meropenem demonstrated an even greater degree of synergistic killing than ertapenem combined with meropenem (7). Furthermore, we demonstrated that all three beta-lactams were needed to achieve synergistic activity and that the effect could reduce CFU per milliliter by at least 4 log₁₀ in 58.3%. Motivated by these results, we sought to determine whether this effect was unique to KPC-producing *K. pneumoniae* or whether it could be found in other serine CPEs. To establish specificity of the synergy, we used NDM-1 producers as controls.

In isolates from patient specimens collected throughout Québec, Canada, we used time-kill assays to evaluate for synergy between piperacillin-tazobactam (piperacillin, Sigma-Aldrich, St. Louis, MO, USA; tazobactam, Cayman Chemicals, Ann Arbor, MI, USA) and meropenem (Fresenius Kabi, ON, Canada). Briefly, isolates were confirmed to be unique via pulsed-field gel electrophoresis (8) and to harbor the KPC, OXA-48-like, or NDM-1 genes by PCR at the Laboratoire de santé publique du Québec, our provincial reference laboratory. This laboratory tests for carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{NMC}, *bla*_{SME}, *bla*_{OXA-48 family}, and *bla*_{GES}) employing an in-house method that uses PCR techniques and primers based on previously published methods (9, 10).

Citation Lawandi A, Leite GC, Cheng MP, Lefebvre B, Longtin J, Lee TC. 2021. Assessment of piperacillin-tazobactam-meropenem synergy against serine carbapenemase-producing *Enterobacterales* using time-kill assays. *Antimicrob Agents Chemother* 65:e02317–20. <https://doi.org/10.1128/AAC.02317-20>.

Copyright © 2021 American Society for Microbiology. All Rights Reserved.

Address correspondence to Alexander Lawandi, alexander.lawandi@nih.gov.

Received 3 November 2020

Returned for modification 5 December 2020

Accepted 17 December 2020

Accepted manuscript posted online 19 January 2021

Published 18 March 2021

TABLE 1 Reduction in log₁₀ CFU/ml of combinations relative to single most active agent

Isolate no.	Species	Carbapenemase type	MIC (mg/liter) ^b of:		Log ₁₀ reduction (CFU/ml) ^c with:			
			MEM	PTZ	MEM (8 mg/liter)	1 × MEM MIC	0.50 × MEM MIC	0.25 × MEM MIC
1	<i>E. coli</i>	KPC	4	>128/4		6	8	8
2 ^a	<i>E. coli</i>	KPC	8	>128/4	3			
3 ^a	<i>E. coli</i>	KPC	16	>128/4	2			
4	<i>E. coli</i>	KPC	2	128/4		5	5	6
5	<i>E. coli</i>	KPC	2	>128/4		4	8	10
6	<i>E. coli</i>	KPC	4	>128/4		4	5	7
7	<i>E. coli</i>	KPC	2	>128/4		2	2	2
8	<i>E. coli</i>	KPC	2	>128/4		5	6	8
9	<i>E. coli</i>	KPC	2	>128/4		7	5	1
10 ^a	<i>E. coli</i>	KPC	8	>128/4	1			
11	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		4	2	1
12	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		4	8	4
13 ^a	<i>K. pneumoniae</i>	OXA-48-like	16	>128/4	2			
14	<i>K. pneumoniae</i>	OXA-48-like	1	>128/4		5	5	-
15	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		7	10	2
16	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		8	1	1
17	<i>K. pneumoniae</i>	OXA-48-like	4	>128/4		5	3	6
18	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		7	2	1
19	<i>K. pneumoniae</i>	OXA-48-like	0.5	>128/4		5	6	1
20 ^a	<i>K. pneumoniae</i>	OXA-48-like	16	>128/4	3			
21 ^a	<i>K. pneumoniae</i>	NDM-1	16	>128/4	0			
22 ^a	<i>K. pneumoniae</i>	NDM-1	>32	>128/4	0			
23 ^a	<i>K. pneumoniae</i>	NDM-1	16	>128/4	0			
24 ^a	<i>K. pneumoniae</i>	NDM-1	>32	>128/4	0			

^aSynergy with meropenem 8 mg/liter was tested because the MICs for the isolates were at the limit of achievable *in vivo* meropenem concentrations in blood (see text for details).

^bMEM, meropenem; PTZ, piperacillin-tazobactam.

^cLog₁₀ reduction at 24 h relative to MEM monotherapy in presence of PTZ 16/4 mg/liter.

MICs were determined by broth microdilution per CLSI methodology (11), and the potencies of the antibiotics were confirmed serially over time using MIC determination of the quality control strain (12). The antibiotic concentrations used in the assays were selected in two ways. First, the concentrations were chosen to reflect those achievable *in vivo* in critically ill patients using optimal dosing strategies, i.e., 16/4 mg/liter for piperacillin-tazobactam and 8 mg/liter for meropenem (13, 14). Second, for organisms in which the meropenem MIC was <8 mg/liter, the time-kill assays were performed using concentrations of meropenem relative to the isolate's MIC (1 × MIC, 0.5 × MIC, and 0.25 × MIC). Each bacterial isolate was suspended in cation-adjusted Mueller-Hinton broth with piperacillin-tazobactam to achieve an initial bacterial concentration of 10⁶ CFU/ml. These suspensions were then incubated at 37°C and continuously mixed on a rotator. After 30 minutes of incubation, meropenem was added to the suspension in a minimal volume. The final concentration of meropenem was based on the meropenem MIC as described above. Subsequently, the suspension was reincubated at 37°C and continuously mixed, with colony counts performed per standard technique at 0.5, 4, 8, and 24 h. Synergy was defined as a ≥2-log₁₀-CFU/ml reduction at 24 h of exposure to the antibiotic combination compared with the single most active antibiotic, with the number of surviving organisms in the presence of the combination at >2 log₁₀ CFU/ml below the starting inoculum. Furthermore, it was required that piperacillin-tazobactam not appreciably affect the growth curve when used alone.

A total of 24 strains were included in this study, including 10 KPC-producing *Escherichia coli* isolates, 10 OXA-48 family-producing *K. pneumoniae* isolates, and 4 NDM-1-producing *K. pneumoniae* isolates. The MICs of the strains to piperacillin-tazobactam and meropenem and the results of the time-kill assays are listed in Table 1. Representative time-kill curves are shown in Fig. 1 for tested KPC producers and in Fig. 2 for tested OXA-48-like producers. The median MIC to meropenem was

FIG 1 Time-kill assay results for isolate 1. Meropenem (MER) concentrations were as follows: 1 mg/ml ($0.25 \times \text{MIC}$) (A), 2 mg/ml ($0.5 \times \text{MIC}$) (B), 4 mg/ml ($1 \times \text{MIC}$) (C). PTZ, piperacillin-tazobactam.

FIG 2 Time-kill assay results for isolate 19. Meropenem (MER) concentrations were as follows: 0.125 mg/ml (0.25 × MIC) (A), 0.25 mg/ml (0.5 × MIC) (B), 0.5 mg/ml (1 × MIC) (C). PTZ, piperacillin-tazobactam.

3 mg/liter (interquartile range [IQR], 2 to 7 mg/liter) for the KPC-producing isolates and 0.5 mg/liter (IQR, 0.5 to 3.25 mg/liter) for the OXA-48-like-producing isolates. The MICs for the NDM-1 isolates to meropenem were either 16 or >32 mg/liter. For all isolates, the growth curves in the presence of piperacillin-tazobactam alone approximated those for the growth control. Synergy between piperacillin-tazobactam and meropenem was detected for 7 of 10 KPC-producing isolates and 9 of 10 OXA-48-like-producing isolates. No synergy was detected for the NDM-1-producing controls. For the 5 of 7 KPC-producing *E. coli* isolates whose MICs to meropenem were <8 mg/liter, the addition of piperacillin-tazobactam to meropenem at $0.25\times$ the MIC demonstrated a $\geq 6\text{-log}_{10}\text{-CFU/ml}$ reduction at 24 h compared with meropenem monotherapy. The $\log_{10}\text{-CFU/ml}$ reduction for the combination relative to the single most active agent was less as meropenem monotherapy approached the MIC, indicating increasing activity of the monotherapy; however, synergy could still be demonstrated. A similar pattern was not observed with the OXA-48-like producers. Most of these isolates were susceptible to meropenem alone but also showed synergy between piperacillin-tazobactam and meropenem when the concentration of the meropenem was $0.5\times$ or equal to the MIC. OXA-48-producing isolates did not demonstrate synergy between agents at $0.25\times$ the meropenem MIC.

As antimicrobial resistance continues to evolve, it becomes critical to carefully evaluate existing drugs and determine how best to apply them, both alone and in combination. Although several carbapenemase inhibitors have come to market in the United States and Europe, there has already been a ceftazidime-avibactam shortage worldwide (15), and these drugs are not readily available or are prohibitively expensive in many countries. For instance, no carbapenemase inhibitor products are currently available in Canada. We previously demonstrated that the combination of piperacillin-tazobactam and meropenem showed synergistic killing against KPC-producing *K. pneumoniae* isolates. Here, we demonstrate that this effect is seen across a range of serine carbapenemase-producing organisms.

The biological mechanism for the increased activity of the drug combination against the serine carbapenemase producers remains unknown. Our results may suggest that the synergy observed involves inhibition of the carbapenemase enzymes themselves, given the specificity to the serine carbapenemases and the lack of efficacy against the metallo-beta-lactamase producers. Because the serine carbapenemase enzyme has a very wide range of beta-lactam substrates, it is plausible that the mechanism of synergy would likely involve saturation of the enzymatic active site such that the bactericidal beta-lactams can exert their effect. This is the proposed mechanism for double-carbapenem therapy (16). However, in our previous work, we showed that the synergy required the specific beta-lactams of meropenem, piperacillin, and tazobactam together; substituting for other carbapenems or cephalosporins resulted in reduction or removal of synergy (7). Whereas this could reflect specifics of the enzyme-substrate interactions and kinetics that favor the meropenem-piperacillin-tazobactam combination, this remains speculative because it has not been specifically investigated to date. We also acknowledge that there are numerous other commonalities between the two species tested, i.e., *E. coli* and *K. pneumoniae*. Consequently, there could be specific penicillin-binding protein inhibition or off-target effects that may also explain the results, although we would have expected similar synergy in the NDM-1 producers. One limitation of this study is that the control organisms, the NDM-1 producers, have much higher MICs to meropenem than the OXA-48-like and KPC producers. The higher potency of the enzymes for the substrates might be considered another limitation to synergy. Further work with a wider range of isolates will be required. We also acknowledge that using time-kill assays alone cannot characterize the causes of antibacterial synergy. The lack of universality of the synergy among the serine carbapenemase-producing isolates shown here and in our previous work suggests that some of the isolates possessed other resistance elements that could obviate the effectiveness of the combination. Further genetic characterization of these isolates is under way. Similarly,

Enterobacter species are common producers of carbapenemases, particularly of KPC enzymes (17). However, members of this genus also frequently contain a number of Ambler class C enzymes, as well as various metallo-beta-lactamases. Because the theorized mechanism of synergy involves the suicide inhibition of the carbapenemase enzyme, the addition of other enzymes that may bind to meropenem, piperacillin, and tazobactam may preclude the bactericidal synergy observed here. Consequently, we did not test these species in this series of experiments because of the potential confounding of results.

In conclusion, our results further support the potential for combining piperacillin-tazobactam with meropenem in use against serine CPEs. Further *in vivo* studies and studies providing a mechanistic explanation for the combination are warranted.

ACKNOWLEDGMENTS

This research was supported in part by the Intramural Research Program of the NIH Clinical Center.

The findings and conclusions in this study are those of the authors and do not necessarily represent the official position of the National Institutes of Health.

REFERENCES

1. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P, European Network on Carbapenemases. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 18:413–431. <https://doi.org/10.1111/j.1469-0691.2012.03821.x>.
2. Temkin E, Fallach N, Almagor J, Gladstone BP, Tacconelli E, Carmeli Y. 2018. Estimating the number of infections caused by antibiotic-resistant *Escherichia coli* and *Klebsiella pneumoniae* in 2014: a modelling study. *Lancet Global Health* 6:e969–e979. [https://doi.org/10.1016/S2214-109X\(18\)30278-X](https://doi.org/10.1016/S2214-109X(18)30278-X).
3. van Duin D, Doi Y. 2017. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 8:460–469. <https://doi.org/10.1080/21505594.2016.1222343>.
4. Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, Simmer PJ. 2017. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. *Clin Infect Dis* 64:257–264. <https://doi.org/10.1093/cid/ciw741>.
5. Dautzenberg MJD, Wekesa AN, Gniadkowski M, Antoniadou A, Giamarellou H, Petrikos GL, Skiada A, Brun-Buisson C, Bonten MJM, Derde LPG, Mastering Hospital Antimicrobial Resistance in Europe Work Package Study Team. 2015. The association between colonization with carbapenemase-producing Enterobacteriaceae and overall ICU mortality: an observational cohort study. *Crit Care Med* 43:1170–1177. <https://doi.org/10.1097/CCM.0000000000001028>.
6. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. 2014. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerg Infect Dis* 20:1170–1175. <https://doi.org/10.3201/eid2007.121004>.
7. Lawandi A, Leite G, Cheng MP, Lefebvre B, Longtin J, Lee TC. 2019. In vitro synergy of β -lactam combinations against KPC-producing *Klebsiella pneumoniae* strains. *J Antimicrob Chemother* 74:3515–3520. <https://doi.org/10.1093/jac/dkz389>.
8. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33:2233–2239. <https://doi.org/10.1128/JCM.33.9.2233-2239.1995>.
9. Lee TD, Adie K, McNabb A, Purych D, Mannan K, Azana R, Ng C, Tang P, Hoang LMN. 2015. Rapid detection of KPC, NDM, and OXA-48-like carbapenemases by real-time PCR from rectal swab surveillance samples. *J Clin Microbiol* 53:2731–2733. <https://doi.org/10.1128/JCM.01237-15>.
10. Swayne RL, Ludlam HA, Shet VG, Woodford N, Curran MD. 2011. Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo- (class A and D) carbapenemases in Enterobacteriaceae. *Int J Antimicrob Agents* 38:35–38. <https://doi.org/10.1016/j.ijantimicag.2011.03.010>.
11. CLSI. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—11th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
12. Leite G, Lawandi A, Cheng MP, Lee TC. 2019. Stability of biological activity of frozen β -lactams over time as assessed by time-lapsed broth microdilutions. *Antibiotics* 8:165. <https://doi.org/10.3390/antibiotics8040165>.
13. Burgess DS, Waldrep T. 2002. Pharmacokinetics and pharmacodynamics of piperacillin/tazobactam when administered by continuous infusion and intermittent dosing. *Clin Ther* 24:1090–1104. [https://doi.org/10.1016/S0149-2918\(02\)80021-2](https://doi.org/10.1016/S0149-2918(02)80021-2).
14. Cojutti P, Sartor A, Righi E, Scarparo C, Bassetti M, Pea F. 2017. Population pharmacokinetics of high-dose continuous-infusion meropenem and considerations for use in the treatment of infections due to KPC-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 61:e00794–17. <https://doi.org/10.1128/AAC.00794-17>.
15. U.S. Food and Drug Administration. FDA drug shortages. [https://www.accessdata.fda.gov/scripts/drugshortages/dsp_ActiveIngredientDetails.cfm?AI=AVYCAZ%C2%AE%20\(ceftazidime%20and%20avibactam\)%20for%20Injection,%202%20grams/0.5%20grams&st=c](https://www.accessdata.fda.gov/scripts/drugshortages/dsp_ActiveIngredientDetails.cfm?AI=AVYCAZ%C2%AE%20(ceftazidime%20and%20avibactam)%20for%20Injection,%202%20grams/0.5%20grams&st=c). Accessed 29 October 2020.
16. Bulik CC, Nicolau DP. 2011. Double-carbapenem therapy for carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 55:3002–3004. <https://doi.org/10.1128/AAC.01420-10>.
17. van Duin D, Arias CA, Komarow L, Chen L, Hanson BM, Weston G, Cober E, Garner OB, Jacob JT, Satlin MJ, Fries BC, Garcia-Diaz J, Doi Y, Dhar S, Kaye KS, Earley M, Hujer AM, Hujer KM, Domitrovic TN, Shropshire WC, Dinh A, Manca C, Luterbach CL, Wang M, Paterson DL, Banerjee R, Patel R, Evans S, Hill C, Arias R, Chambers HF, Fowler VG, Kreiswirth BN, Bonomo RA, Multi-Drug Resistant Organism Network Investigators. 2020. Molecular and clinical epidemiology of carbapenem-resistant Enterobacterales in the USA (CRACKLE-2): a prospective cohort study. *Lancet Infect Dis* 20:731–741. [https://doi.org/10.1016/S1473-3099\(19\)30755-8](https://doi.org/10.1016/S1473-3099(19)30755-8).